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The Association Between Elevated Hippocampal Glutamate Levels and Cognitive Deficits in Epilepsy

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THE ASSOCIATION BETWEEN ELEVATED HIPPOCAMPAL GLUTAMATE LEVELS AND COGNITIVE DEFICITS IN EPILEPSY.

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The purpose of this study was to investigate the association between extracellular basal hippocampal glutamate levels and cognitive function in epileptic patients. We used the zero-flow microdialysis method to measure the extracellular concentrations of glutamate in the epileptogenic and non-epileptogenic hippocampus of 23 awake epileptic patients during the interictal period. All patients underwent extensive neuropsychological testing to assess cognitive functioning prior to probe implantation. Basal glutamate levels in the epileptogenic hippocampus were significantly higher than the non-epileptogenic hippocampus (mean, 11.96 micromolar (μM) versus 2.92 μM, respectively). Elevated basal glutamate levels in the epileptogenic hippocampus correlated with decreased scores on the Verbal Selective Reminding Test (V-SRT) (R^2 = 0.36, p = 0.0244). When controlling for MRI-detected hippocampal atrophy within epileptogenic regions, elevated basal glutamate levels within atrophic hippocampus correlated with decreased cognitive functioning measured by both the V-SRT (R^2 = 0.7764, p = 0.0204) and Performance Intelligence Quotient (PIQ) (R^2 = 0.7324, p = 0.0297), but not within non-atrophic hippocampus (V-SRT: R^2 = 0.1013, p = 0.4424; PIQ: R^2 = 0.2303, p = 0.2288). These data suggest that elevated basal glutamate levels in the epileptogenic hippocampus may be implicated in the pathogenesis of hippocampal atrophy and may contribute to impaired cognitive functioning involving verbal memory and visual-spatial skills in patients with temporal lobe epilepsy.

Introduction

Glutamate as a Principal Neurotransmitter and Excitotoxin

Glutamate is the principal excitatory neurotransmitter in the brain, and it is thought to mediate learning and memory (1). Glutamate was first suggested to play a role in central nervous system metabolism in 1943, when Price and colleagues (2) reported the successful treatment of petit mal seizures with orally administrated glutamate. Although this claim regarding the therapeutic effect of glutamate could not be substantiated by later studies, the report sparked interest in glutamate's involvement in brain function and fostered an advent of research in this field (1). The excitatory effect of glutamate on the central nervous system was first elucidated by Hayashi and colleagues (3), when the application of glutamate to the cortex of dogs and humans led to "clonic convulsions." A plethora of physiologic and biochemical evidence has since implicated glutamate as a fundamental excitatory neurotransmitter involved in clinically important neuroanatomical networks, including the hippocampal pathways of learning and memory (4).

The extracellular concentration of glutamate in the healthy brain is normally kept very low due to efficient glutamate re-uptake mechanisms. Meldrum and colleagues (5) report that although glutamate is found in high intracellular concentrations throughout the mammalian brain, the baseline level of extracellular glutamate approximates one micromolar (μM). During neuronal transmission, glutamate is released into the synaptic cleft and the concentration of the neurotransmitter rises transiently to approximately one millimolar (mM) for the maximal activation of glutamate receptors. Efficient neuronal and glial sodium-dependent excitatory amino acid transporters (EAATs) quickly remove

glutamate from the synaptic cleft to return extracellular concentrations to normally low baseline levels (6).

In pathological conditions, abnormal elevation in extracellular glutamate levels may result in excitotoxic cell damage leading to neuronal cell death and impaired function (7). In 1971, Olney and colleagues (8) introduced the term "excitotoxin" after demonstrating that systemic administration of glutamate resulted in toxic damage to the central nervous system, and that the excitatory properties of amino acids correlated positively with their ability to produce neurotoxic damage. Both *in vivo* and *in vitro* studies (7, 9, 10) demonstrated that excitotoxicity occurs following abnormally elevated exposure to glutamate. The pathogenesis of glutamate-induced excitotoxic cell death occurs via an acute sodium-dependent phase followed by a delayed calcium-dependent phase (11). The acute component occurs within minutes of glutamate binding to the *N*-methyl-D-aspartic acid (NMDA) receptor, during which the sodium influx is associated with passive influx of water and chloride with resultant neuronal dendritic swelling. The delayed component occurs hours post-exposure and is triggered by excessive calcium influx, which produces a cascade-like effect leading to cell death (11, 12). This excessive calcium influx has been implicated in the glutamate-induced injury of hippocampal neurons (13). Moreover, Mattson and colleagues (14) report that the hippocampal subpopulation of pyramidal cells in rats are particularly vulnerable to glutamate-induced neurotoxicity when compared to bipolar or stellate cells, and demonstrate that pyramidal cells in the CA1 hippocampal region show greatest vulnerability to excitotoxic damage, followed by cells in the CA3 region, the CA2 region, and finally the dentate gyrus.

The Hippocampus and Memory

The hippocampus is a pivotal structure of the medial temporal lobe and its functional integrity is requisite for learning and memory. Compelling evidence in support of this comes from the famous case report of patient H.M. who underwent bilateral hippocampectomy for treatment of medically refractory epilepsy, with resultant post-surgical profound anterograde amnesia (15) despite preservation of intelligence. While this memory impairment followed bilateral hippocampal loss, similar deficits have been reported in patients undergoing unilateral hippocampal resection with prior concomitant damage to the contralateral hippocampus, creating the functional equivalent of bilateral hippocampal lesions (16). However, memory impairments are less severe in patients undergoing unilateral hippocampectomy with intact contralateral temporal lobe function (17). Furthermore, memory impairments following unilateral hippocampal resection are dependent on surgical lateralization; verbal memory is more severely impaired following resection of the dominant (usually left) hippocampus, while visual-spatial learning deficits occur following non-dominant (usually right) hippocampectomy (17). These lesion studies have generated a body of evidence suggesting that the hippocampus is responsible for memory operations in the medial temporal lobe.

Although hippocampal participation in cognition is well recognized, the precise role it plays in memory is still being elucidated. Memory is divided into two broad classes; declarative (explicit) memory refers to the conscious recollection of facts and is subdivided into semantic memory (one's general knowledge base) and episodic memory (one's recollection of personal experiences), while non-declarative (implicit) memory involves the non-conscious recollection of skills and comprises procedural learning,

perceptual priming, and conditioning (18). Non-declarative memory functions independently of the hippocampus, as Milner (19, 20) demonstrates that this form of memory may be acquired, maintained, and retrieved even by patients who are profoundly amnesic as a result of hippocampal damage. In contrast, declarative memory is critically dependent on hippocampal function, although there is considerable debate regarding whether the hippocampus serves as either the temporary storage site of semantic and episodic information awaiting consolidation (21) or the permanent storage site of episodic memory as multiple memory traces (22, 23). Moser and Moser (24) review numerous studies investigating the use of spatial memory for navigation in both rodents and humans, which collectively suggest the hippocampus is critically involved during the encoding and retrieval of spatial memory. Moreover, isolated damage to the human hippocampus has been reported to produce global deficits of declarative memory, with greater disruptions in episodic memory than semantic memory (24). Finally, functional imaging studies in healthy patients demonstrate hippocampal activation during encoding of verbal and visual information (25). This growing body of evidence from imaging and task-learning studies in both animal and human models clearly suggests that the hippocampus is specifically involved in the encoding process of verbal and visual-spatial memory.

Separate anatomical sub-regions of the hippocampus are differentially engaged in the learning process, however the exact role these different segments play has not yet been fully elucidated. Moser and Moser (24) suggest that the anterior one-third of the hippocampus is functionally distinct from the posterior two-thirds. Based primarily on animal studies, these investigators propose that episodic memory depends on the

posterior two-thirds of the hippocampus alone, while the anterior hippocampus functions in emotional control systems. In contrast, Ros and colleagues (26) demonstrate via autoradiogram densitometry that the hippocampus is activated heterogeneously along its anterior-posterior axis during spatial learning tasks in mice. The pattern of metabolic activation reveals increased activity in the intermediate and posterior CA1 region during early acquisition, and in the anterior and posterior CA1 region and the anterior dentate gyrus during late acquisition (26). In a meta-analysis of positron emission tomography (PET) imaging studies, Lepage and colleagues (27) report the anterior hippocampus is engaged during the encoding of episodic memory while the posterior region becomes activated during retrieval. Furthermore, human functional magnetic resonance imaging (fMRI) studies demonstrate that processing novel information involves the anterior hippocampus, while processing familiar material utilizes the posterior hippocampus (28-30). Moreover, fMRI studies in primates suggest that the posterior hippocampus mediates spatial information, in contrast to anterior regions which, in animals, appear to direct non-spatial information processing (31). Similar fMRI studies in healthy humans demonstrate significant activation of the posterior hippocampus during verbal learning as compared to the anterior hippocampus (25, 32). Taken together, this evidence suggests the anterior hippocampus is predominantly involved in the encoding process of novel information, while the posterior hippocampus is responsible for retrieval, processing familiar information, and the mediation of verbal and spatial information.

The cellular basis for the storage of information is thought to depend upon a form of synaptic plasticity known as long-term potentiation (33). Long-term potentiation (LTP) upholds that repeated neuronal stimulation leads to synaptic sensitization, such that

a constant level of stimulus evokes an augmented post-synaptic response which may last for days to weeks. This strengthening of the synaptic connection between neurons promotes efficacy of information processing, which is appropriate for the acquisition and storage of memory (34). The molecular mechanisms of LTP are currently being elucidated; it remains controversial whether this synaptic strengthening is maintained by an augmented release of neurotransmitter from the pre-synaptic cell, an increased number of receptors in the post-synaptic cell, or a combination of both (35). Moreover, it is debated whether these molecular mechanisms underlying LTP are the same as those responsible for memory (36), however there is considerable evidence suggesting this is the case. LTP has been described in various hippocampal pathways, including the Schaffer collateral – CA1 synapse and the perforant path – dentate granule cell synapse (18, 37). It is well documented that LTP is mediated by the interaction of glutamate with its NMDA receptor (38). Beck and colleagues (37) report numerous studies demonstrating that antagonists of NMDA receptors prevent induction of LTP and result in spatial learning deficits in rodents. Furthermore, electrophysiological analysis of hippocampal specimens from patients with temporal lobe epilepsy (TLE) has revealed that activity-dependent synaptic plasticity for information storage similar to the rodent model is also present within humans (37). These findings demonstrate that the excitatory properties of glutamate within hippocampal pathways are crucial to learning and memory.

Impaired Cognition in Temporal Lobe Epilepsy

The hippocampal model of memory is exemplified by patients afflicted with temporal lobe epilepsy (TLE), in which hippocampal pathology is associated with deficits in learning in memory. The epileptogenic focus in this form of epilepsy involves the medial temporal lobe, evidenced by studies demonstrating the localization of abnormal electrical foci to this region, as well as data from postoperative pathology specimens revealing histological abnormalities of the temporal lobe (39). TLE has long been associated with cognitive deficits, particularly with impairments in intelligence quotient (IQ) and verbal memory associated with long duration of epilepsy and frequent tonic-clonic seizures (40). Multiple neuropsychological assessment tools have been used to quantify cognitive function specifically related to learning and memory in these patients. Briefly, the Wechsler Adult Intelligence Scale (WAIS) provides measures of general intellectual functioning broadly categorized into Verbal Intelligence Quotient (VIQ) and Performance Intelligence Quotient (PIQ); the Full Scale Intelligence Quotient (FSIQ) is a composite of the VIQ and PIQ. The FSIQ is a broad-spectrum measure of cognition that is mediated by global cortical processes; the VIQ and PIQ divide the FSIQ into verbally-mediated and nonverbally-mediated components, respectively. The Verbal Selective Reminding Test (V-SRT) is designed to measure verbal learning and memory during a multiple-trial list-learning task, which is mediated by dominant (usually left) hippocampal function (41). An alternate format exists which measures spatial memory, referred to as the Visual-Motor Selective Reminding Test (VM-SRT), and depends on non-dominant (usually right) hippocampal function.

Verbal memory impairments associated with TLE have been specifically correlated to both pyramidal cell loss and MRI-detected hippocampal atrophy. First, Sass and colleagues (42-46) examined the relationship between neuronal counts of pyramidal cells in various hippocampal subfields and neuropsychological measures. They demonstrated that neuronal loss in the CA3 and hilar regions is associated with verbal memory impairment measured both by the verbal selective reminding test (V-SRT) (42) and the intracarotid amobarbital procedure (IAP), in which one hippocampus is anesthetized to permit independent testing of the contralateral temporal lobe (43). Subsequent studies further elucidated that decreased pyramidal cell density correlates with verbal memory impairment measured specifically by the V-SRT but not to overall verbal intellectual functioning as measured by the VIQ (44), supporting the hypothesis that memory impairment results from hippocampus-specific cell loss as opposed to global dysfunction of the temporal neocortex. Furthermore, the extent of hippocampal cell loss is correlated with the degree of verbal impairment in patients both without (45) and with (46) structural lesions. Second, improvements in neuroimaging have permitted pre-surgical detection of mesial temporal sclerosis (MTS), which is the most common pathological finding of TLE. MTS is characterized by severe neuronal loss and gliosis, and its signature on MRI includes atrophy of the hippocampus on T1-weighted images and increased signal intensity on T2-weighted images (47). This MRI-detected hippocampal pathology in TLE patients has been associated with verbal memory impairment. Westerveld (47) reports the correlation between hippocampal volume loss and asymmetry of memory scores during the intracarotid amobarbital procedure (IAP); the difference in performance between right and left hemispheres indicates impaired

functioning of one hippocampus with preservation of contralateral performance. Moreover, hippocampal volume loss correlates with deficits in baseline neuropsychological functioning, particularly of verbal learning and recall. Lencz and colleagues (48) report that among TLE patients with left-sided seizure foci, MRI-determined left hippocampal volume correlates with Wechsler logical memory percent retention scores, and left temporal lobe measurements correlate with performance on the V-SRT. Kilpatrick and colleagues (49) report a strong association between the degree of MRI-detected left hippocampal atrophy and severity of verbal memory and verbal memory retention deficits measured by the Rey auditory verbal learning task. Taken together, these data correlating both decreased pyramidal cell density and hippocampal atrophy with impaired verbal memory in temporal lobe epilepsy patients clearly demonstrate the necessity of cellular and macroscopic hippocampal integrity for proper cognitive function.

Patients with Temporal Lobe Epilepsy

Epilepsy is a common disorder affecting approximately 15 per 1000 people (50) with resultant significant morbidity and mortality. Mesial temporal lobe epilepsy (MTLE) is the most common form of human epilepsy (51), and its underlying pathology is most often mesial temporal sclerosis (MTS), hallmarked by hippocampal neuronal loss and gliosis (52). MTLE is resistant to antiepileptic medications in approximately 75% of cases (52). Significant disability has been reported in such patients, including depression in approximately 50% of cases with suicidal ideation approaching 20%, and psychosis occurring 6- to 12-fold more frequently than healthy counterparts (53). Further morbidity

and mortality results from seizure injury (including fractures, burns, and death from drowning and driving), in addition to death from status epilepticus, suicide, and sudden unexplained death in epilepsy patients (SUDEP) (53). Moreover, the duration and severity of epilepsy are associated with cognitive dysfunction, including significant verbal memory decline (40, 54) and impairments of global intelligence measured by the WAIS-R (40, 55), which contribute to decreased quality of life in these patients. However, MTLE is surgically remediable by anteromesial temporal resection in 70 to 90% of patients (51, 52, 56). A landmark randomized controlled trial of temporal-lobe surgery for medically refractory TLE demonstrated that 58% of the surgical group was seizure free at one year postoperatively as compared to 8% in the medical group, with improvements in quality of life reported for seizure-free patients (57). Although the cost incurred through preoperative evaluation and surgical resection approximates $100,000 (56), it is comparable to the expense of antiepileptic drug therapy, medical intervention for illness secondary to treatment failure, laboratory studies, office visits, and lost income from unemployment. Furthermore, surgical resection in the treatment of medically refractory epilepsy may improve quality of life and permit the patient to become a functional member of society.

Microdialysis Measurements of the Glutamate Levels in the Epileptogenic Hippocampus

The technique of microdialysis has been applied to patients with refractory medical temporal lobe epilepsy undergoing intracranial depth electrode monitoring to identify seizure focus for possible resection. In this technique, microdialysis catheters are implanted into the brain region of interest so that extracellular brain fluid may be sampled

and analyzed for composition and concentrations of neurotransmitters (58, 59). Chemical substrates within the brain's interstitial fluid diffuse down a concentration gradient across a semi-permeable dialysis membrane into the perfusion fluid inside the catheter. The perfusion fluid is then collected and analyzed (58, 59).

Microdialysis techniques have been employed to study animal models of epilepsy, revealing an association between seizure activity and elevated glutamate levels (60-63). Ueda and colleagues (60) demonstrated an increase in extracellular hippocampal glutamate concentrations during the interictal period of freely-moving rats with chronic kainic-acid induced seizures. Engstrom and colleagues (61) found spontaneous interictal glutamate elevations up to 8 times the basal level in 80% of rats with iron-induced chronic focal epilepsy. Smolders and colleagues (62) report that during pilocarpine-induced limbic convulsions in the freely moving rat, glutamate levels are significantly increased in the hippocampus.

Similarly, several human microdialysis studies report that glutamate levels in the hippocampus of TLE patients may be elevated to neurotoxic ranges (64-70). Early intracerebral microdialysis studies revealed a dramatic increase of extracellular glutamate during the onset and occurrence of seizures (64, 65). Wilson and colleagues (66) report that the glutamate increase during seizures in the human hippocampus is similar to the glutamate rise seen in the chronic kainate rat model of epilepsy. In patients undergoing anteromesial temporal resection, microdialysis recovery of glutamate from spontaneously epileptiform hippocampus was significantly greater than glutamate recovery from non-epileptiform hippocampus (67, 68). A recent microdialysis study by Cavus and colleagues (69) comparing epileptogenic and non-epileptogenic hippocampus during

interictal periods demonstrates that while glutamate levels remain low in non-epileptogenic regions, basal extracellular glutamate levels are elevated to potentially neurotoxic ranges in the epileptogenic hippocampus. Moreover, During and Spencer (70) report that extracellular glutamate levels in the epileptogenic hippocampus rise further above baseline immediately preceding seizures and remain elevated for prolonged post-ictal periods as compared to healthy brain, possibly exacerbating neurotoxic damage. Furthermore, extracellular glutamate concentrations have been found to increase significantly during the performance of complex figure memory tasks, and this elevation is both augmented and prolonged in epileptogenic hippocampus, suggesting that the epileptic brain is particularly vulnerable to the cognitive activation-induced glutamate release (71). Recent evidence from microdialysis studies has further demonstrated that elevated basal glutamate levels in the epileptogenic hippocampus are correlated with both decreased neuronal density measured by histological analysis and MRI-detected quantitative hippocampal atrophy (Cavus, unpublished data), suggesting that glutamate elevation to neurotoxic levels may mediate the neuronal cell loss and sclerosis associated with cognitive deficits.

Statement of Purpose

The purpose of this study is to identify the relationship between basal hippocampal glutamate levels and cognitive function in patients with TLE. In brief review, glutamate is the principal excitatory neurotransmitter and levels remain low in healthy brain, however concentrations may rise to neurotoxic levels in the epileptogenic hippocampus (1, 5, 7, 64-70). The hippocampus is responsible for learning and memory (15-17, 24-27), thus impaired hippocampal function in TLE is associated with cognitive deficits (40, 54, 55). Specifically, microdialysis evidence demonstrates that elevated glutamate levels are associated with cell loss and hippocampal atrophy (Cavus, unpublished data), and previous studies have demonstrated that both cell loss and hippocampal atrophy are associated with verbal memory impairment (42-49). We therefore hypothesize that elevated basal glutamate levels in the epileptogenic (and often atrophic) hippocampus will be associated with poorer cognitive function. Further, we hypothesize that elevated basal glutamate levels in the epileptogenic hippocampus are associated particularly with impaired verbal memory measured by a neuropsychological test specific for hippocampal verbal functioning, the verbal selective reminding task, but not with global intelligence measured by the Verbal IQ, Performance IQ, and Full Scale IQ, as these tests are more dependent upon cortical processes and less dependent on memory and hippocampal-specific processes. Conversely, we hypothesize that the low basal glutamate level in healthy non-epileptogenic hippocampus will not be associated with verbal memory deficits.

Methods

Patients

Patients with drug-resistant epilepsy receive a phased evaluation of their illness to localize their seizure focus for possible surgical resection. All patients undergo Phase I clinical evaluation, in which they are admitted to an epilepsy unit for continuous audio-video/electroencephalogram (EEG) monitoring with interictal and ictal scalp recording during at least three typical seizures. Patients also receive brain magnetic resonance imaging (MRI) with measurement of hippocampal volume, interictal and ictal single-photon emission computed tomography (SPECT), interictal positron emission tomography (PET), and extensive neuropsychological assessment. Phase II evaluation consists of the intracarotid amobarbital procedure to determine lateralized memory function and hemispheric dominance for language. Those patients in whom the seizure focus could either not be localized by Phase I and II evaluations or if there was discordant data were offered intracranial EEG monitoring (Phase III study), which involved continuous audiovisual monitoring and EEG recording from a combination of depth, subdural strip, and grid electrodes implanted intracranially in brain regions suspected of being involved in seizure generation. These patients undergoing Phase III evaluation were also invited to participate in the microdialysis study between 2000 and 2005. Patients who consented to the study received implantation of depth electrodes which were coupled to microdialysis probes, allowing for simultaneous electrophysiological recording and sampling of the extracellular fluid. The recordings from the two depth electrode contacts flanking the microdialysis membrane were used to determine whether the microdialysis catheter was within or outside an epileptogenic area. The epileptogenic

area was defined as the site of seizure origin in at least one seizure, whereas the non-epileptogenic sites were either not involved or were only secondarily involved (propagated) during seizures. Epileptogenic probes are defined as microdialysis catheters coupled to electrodes which recorded from epileptogenic areas, while non-epileptogenic probes are those catheters which recorded from non-epileptogenic brain areas.

Twenty-two patients participated in the study. The mean age at the time of the Phase III study was 36.3 years (standard deviation (SD), 12.4 years), with 13 female patients: (mean age ± SD) 36.4 ± 12.1 years and 9 male patients: 36.2 ± 13.7 years. The mean duration of epilepsy was 19.7 years (SD, 12.8 years), with the mean duration of epilepsy for females equal to 18.1 ± 12.9 years and the mean duration for males equal to 22.1 ± 13.1 years (Table 1).

The sample was confined to English-speaking patients without co-morbid medical conditions, 17 years or older, with a Wechsler Adult Intelligence Scale-Revised (WAIS-R) full scale IQ of 70 or greater, and the intracarotid amobarbital procedure demonstrating left hemisphere speech dominance. For patients with multiple intracranial microdialysis catheters, only data from probes located in the anterior hippocampus were included in the study, based on previous reports of differential involvement of the anterior and posterior hippocampus in cognitive processing (24, 26-32). Furthermore, patients were excluded from the study if there was ambiguous probe placement (e.g. at the juncture of white and grey matter), tumor, or diffuse dysplasia at or close to the microdialysis catheters. All patients gave informed consent to the research protocol that was approved by Yale University School of Medicine Human Investigation Committee. Demographic data of participating patients was obtained by this investigator through

medical chart review, and then recorded in a common database. Table 1 summarizes patient information regarding gender, age of seizure onset and duration of epilepsy, probe location, and classification of disease state as epileptogenic versus non-epileptogenic. MRI findings of the hippocampus ipsilateral to the probe are reported, as well as pathology at the probe site following resection if available.

Table 1. Patient Data

Case No.	Sex	Age (yr)	Epilepsy Duration (yr)	Probe Location (no. of probes)	Epileptogenic vs. Non-Epileptogenic	Clinical MRI Findings	Pathology at Probe Site
1	M	53	34.0	L hipp	Epileptogenic	Atrophy	No surgery
2	M	39	12.6	L hipp	Epileptogenic	Atrophy	No surgery
3	M	40	40.3	L hipp	Epileptogenic	Atrophy	No surgery
4	M	17	5.2	R hipp	Epileptogenic	Atrophy	Hipp Sclerosis
5	F	52	17.4	R hipp	Epileptogenic	Atrophy	Hipp Sclerosis
6	F	32	28.3	R hipp	Epileptogenic	Atrophy	Hipp Sclerosis
7	M	50	40.0	L hipp	Epileptogenic	No Atrophy	No surgery
8	F	38	2.0	L hipp	Epileptogenic	No Atrophy	No surgery
9	F	36	33.6	L hipp	Epileptogenic	No Atrophy	No surgery
10	F	49	47.8	L hipp	Epileptogenic	No Atrophy	No cell loss
11	F	46	9.1	L hipp	Epileptogenic	No Atrophy	Mild WM gliosis
12	M	23	23.2	R hipp	Epileptogenic	No Atrophy	No cell loss
13	F	17	8.0	R hipp	Epileptogenic	No Atrophy	No surgery
14	F	52	8.3	L & R hipp (2)	Epileptogenic (1) Non-Epileptogenic (1)	No Atrophy (2)	No surgery
15	M	27	11.0	L hipp	Non-Epileptogenic	Atrophy	No surgery
16	F	27	17.5	L hipp	Non-Epileptogenic	No Atrophy	No surgery
17	M	52	17.4	L hipp	Non-Epileptogenic	No Atrophy	No surgery
18	M	25	15.0	L hipp	Non-Epileptogenic	No Atrophy	No surgery
19	F	19	7.1	L hipp	Non-Epileptogenic	No Atrophy	No surgery
20	F	23	12.0	R hipp	Non-Epileptogenic	No Atrophy	No surgery
21	F	39	26.0	R hipp	Non-Epileptogenic	No Atrophy	No surgery
22	F	43	18.0	R hipp	Non-Epileptogenic	No Atrophy	No surgery

MRI = magnetic resonance imaging; M = male; F = female; L = left; R = right; hipp = hippocampus; WM = white matter.

Neuropsychological Assessment Techniques

Examiners in the Yale University School of Medicine Department of Neuropsychology administered both the WAIS-R to measure Intelligent Quotients (Verbal IQ, Performance IQ, and Full Scale IQ) and the Selective Reminding Tests (Verbal SRT and Visuo-Motor SRT) during the pre-surgical screening of all patients. Patients were treated with therapeutic ranges of anticonvulsant medication and maintained seizure-free during the 12-hour period prior to examination. Neuropsychological test results were obtained by this investigator through a review of patient charts and subsequently compiled into a common database.

The WAIS-R (72) is a core measure of global intellectual functioning in which an examiner asks tests questions and displays puzzles. The patient's responses are recorded in an individual response booklet and scored. The WAIS consists of 6 verbal subtests (Information, Digit Span, Vocabulary, Arithmetic, Comprehension, and Similarities) in addition to 5 performance subtests (Picture Completion, Picture Arrangement, Block Design, Object Assembly, and Digit Substitution). Sub-scores on the Verbal Intelligence Quotient (VIQ) subtests and Performance Intelligence Quotient (PIQ) are combined to determine a composite Full Scale Intelligence Quotient (FSIQ) (41).

The Verbal Selective Reminding Test (V-SRT) (73) consists of a list of 12 unrelated words which are presented to the subject and must be recalled over multiple trials immediately following recitation. The examiner will repeat only those words not recalled by the subject on the previous trial, permitting the assessment of both short-term and long-term memory. Stimulus exposure continues until the patient recalls the entire list on two consecutive trials, or a total of 12 trials have been performed (41). Standard

scoring yields measures of total recall, long-term memory storage, long-term memory retrieval, consistent long-term memory retrieval, and short-term memory retrieval, which are highly correlated (42). Factor analysis by Westerveld and colleagues (74) has demonstrated that these measures constitute a single factor. V-SRT scores are therefore reported as a single factor using a z-score distribution (average = 0, standard deviation = 1), in which a score of 0.0 equals the average of non-epileptic samples studied by Westerveld. Negative scores indicate performance below average, while positive scores are above average (45). The Visual-Motor Selective Reminding Test (VM-SRT) is administered and scored in a similar fashion to the V-SRT, with visual stimuli replacing the list of 12 words and recall assessed through the drawing of stimuli after exposure.

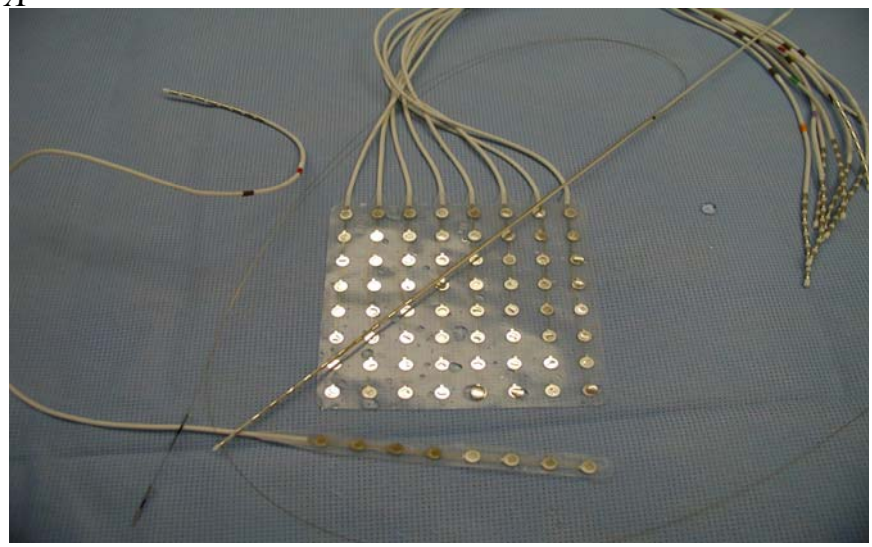
Microdialysis Procedure

Microdialysis probes coupled to depth electrodes (Spencer probe; Ad-Tech Instrument, Racine, WI) were implanted stereotaxically in hippocampal regions suspected of seizure involvement. All surgeries were performed by Drs. Dennis Spencer, Kenneth Vives, and members of the Yale-New Haven Hospital Department of Neurosurgery. The design of the earlier Spencer probe has been modified (75). Briefly, the probes are CMA custom-modified microdialysis probes (CMA/20 concentric flexible probe, 20 kDA membrane pore size, CMA, North Chelmsford, MA) which allow stable flow, recovery, and dialysate collection throughout the duration of intracranial EEG monitoring. The dialysis probe is inserted into a polyurethane/silastic flexible depth-electrode (1 mm i.d., Ad-Tech Instrument Co., Racine, WI), which has perforations between contacts 1 and 2 to allow for fluid exchange with the membrane. The total

diameter of this combination microdialysis/depth electrode (Spencer probe) is 1.85 mm. The probes were sterilized by gamma radiation and flushed with sterile artificial extracellular fluid (AECF) to ensure patency prior to insertion. After surgery, MRI was used to verify the location of the probes and distinguish between anterior and posterior hippocampal placement. (See Fig 1, A-C.)

Fig 1, A-C. Microdialysis catheters prior to, during, and post-surgical implantation. *(A) Subdural grid electrode and Spencer depth electrode with microdialysis catheter prior to intracranial implantation. (B) Intraoperative implantation of depth electrodes with attached microdialysis catheters and placement of subdural grid electrodes into the temporal cortex of a patient undergoing Phase III evaluation. (C) Magnetic resonance image from a patient with one Spencer probe in the right hippocampus, highlighted in red. Depth electrode contacts 1 through 8 are visible. The platinum contacts of the depth electrode generate significant artifact and appear much larger than their actual diameter of 1mm. The microdialysis membrane, which is not visible on magnetic resonance imaging, lies between depth electrode contacts 1 and 2.*

A



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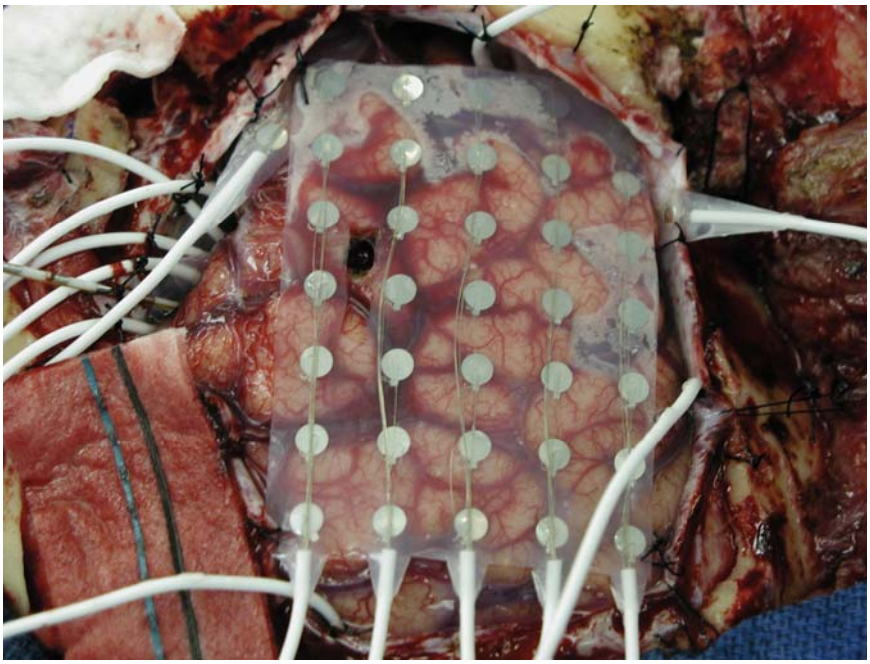

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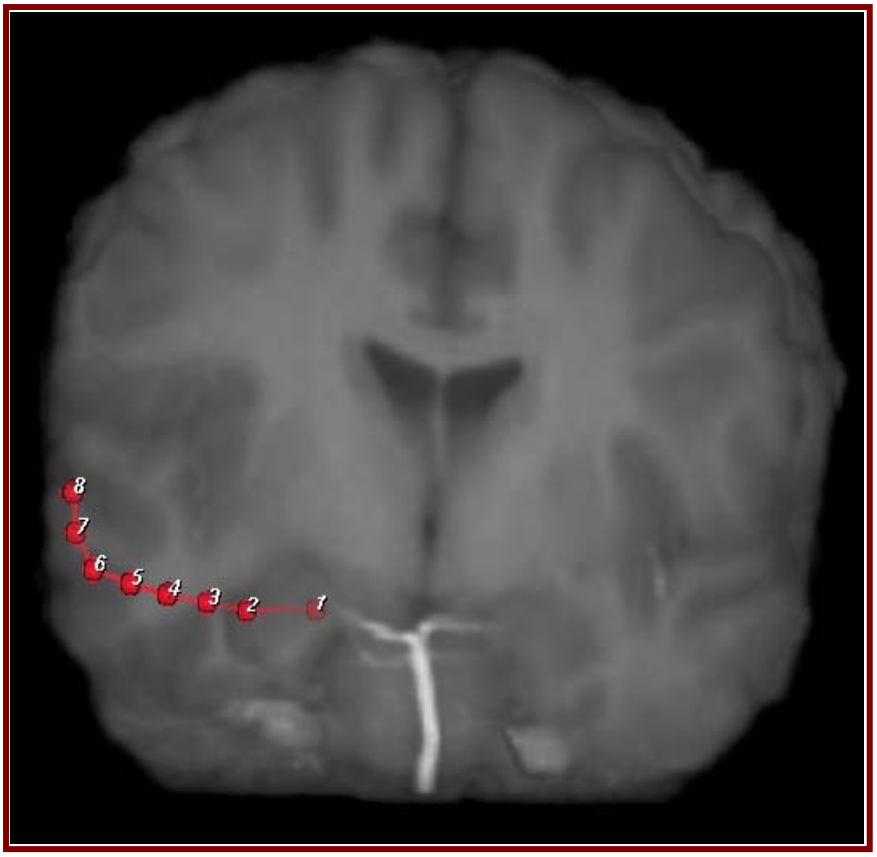


Zero-Flow Study

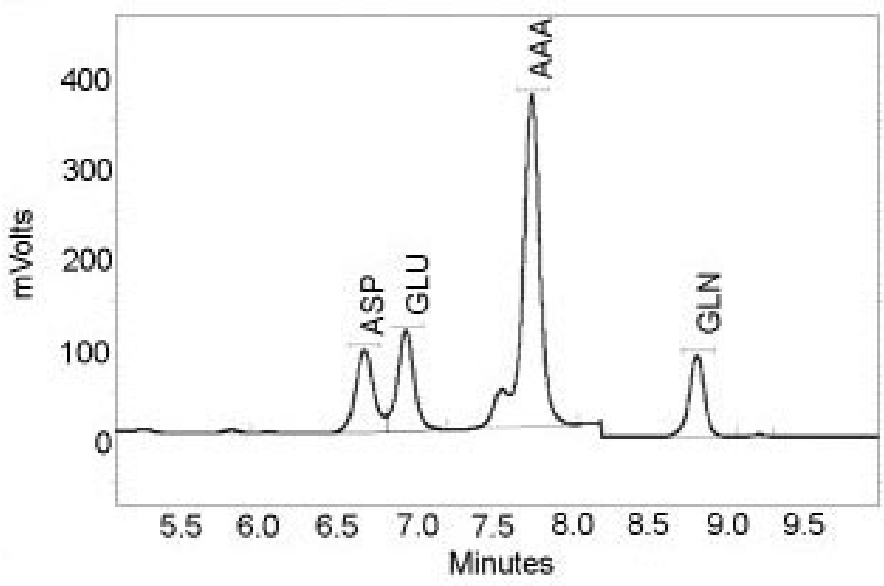
The zero-flow microdialysis method provides an estimate of the true baseline substrate concentration in the extracellular fluid under steady-state conditions (76, 77). The study was conducted 2 to 5 days after surgical probe implantation, at least 6 hours from any intracranially recorded seizure activity and at least 2 hours post-prandially, with the patient quietly resting in the evening, to avoid the effects of acute probe implantation, anesthesia, ictal activity, behavioral stimuli, or food intake (78-80). Patients were maintained on their anti-epileptic medications for the duration of the study. Sterile artificial cerebrospinal fluid (ACSF) (composition: 135mM NaCl, 3mM KCl, 1mM $MgSO_4*7H_2O$, 1.2mM $CaCl_2*2H_2O$ in 1mM sodium phosphate buffer at pH 7.4; Yale-New Haven Hospital Pharmacy) was infused into the hippocampus of awake patients via the inlet tubing on the microdialysis probe using portable CMA107 syringe pumps (CMA, North Chelmsford, MA). Chemical substrates in the hippocampal interstitial fluid diffuse across the dialysis membrane into the artificial CSF within the probe, which is then collected via the outlet tubing on the microdialysis probe into microvials. A flow rate of 2.0 µl/min was initially infused over one hour to reach a steady state, at which point two consecutive 20 µl dialysate samples were collected. The flow rate was then gradually decreased to 1.0, 0.5, and 0.2 µl/min, allowing a period of 60 to 90 minutes after each change in flow rate for equilibration; two consecutive 20 µl dialysate samples were collected at each flow rate. The study was completed over 6 hours, and samples were stored initially over dry ice then placed in a -80ºC freezer for later analysis of neurometabolite concentration using high-performance liquid chromatography. Basal levels of glutamate were determined using regression analysis with fit to second

polynomial order to a flow of zero, which corresponds to steady state. The zero-flow studies were performed by this investigator in conjunction with M. Cassaday, D. Ocame, and S. Forselius.

High Performance Liquid Chromatography Analysis of Glutamate Levels

Glutamate levels were analyzed using high-performance liquid chromatography (HPLC) modified from the method described by Bourdelais and Lakivas (81). Briefly, 1 µl of patient sample is added to 9 µl of an internal standard of alpha-aminoadipic acid (AAA). This mixture is derivatized by adding 20 µl of an O-phthaldialdehyde. After eight minutes, 20 µl of the derivatized sample is injected onto the column (3 um Phase II ODS column, 3.2 x 100 mm cartridge, Bioanalytical Systems, Inc., West Lafayette, IN). The mobile phase consists of 0.1M acetic acid (pH 6.0) with a 12 to 20% acetonitrile gradient at a 1 ml/min flow rate. Within 30 minutes, chromatograms demonstrate adequate separation showing glutamate approximately at 6.9 minutes and AAA at 7.7 minutes (see Fig 2). The excitation and emission wavelengths on the florescence detector (Shimadzu Scientific Instruments, Columbia, MD) are set at 338nm and 425nm, respectively. The sensitivity limit for glutamate is 0.1, based on a signal-to-noise ratio of 10:1. Peak areas of the neurometabolite on chromatograms are then compared with external standards to determine the concentration in the samples using EZCHROME elite software from ESA (Chelmsford, MA). HPLC analysis was kindly performed by M. Cassaday and D. Ocame.

Fig 2. High-performance liquid chromatogram
High-performance liquid chromatogram sample showing the peak separations for aspartate (asp, 2.0μM), glutamate (glu, 1.7μM), the internal standard α-aminoadipic acid (AAA), and glutamine (gln, 80.5μM). The standard curve graph for glutamine and glutamate is shown in the inset. (Reprinted with permission from Cavus et al, Annal Neurol 2005.)



Statistical Analysis

All statistical analyses were performed using JMP 5.0 Statistical Package (SAS Institute Inc., NC). Data of neuropsychological results follow a normal distribution; glutamate data were transformed by log for normalization. Data were analyzed by analysis of variance (ANOVA) in which the neuropsychological variables (VIQ, PIQ, FSIQ, V-SRT, and VM-SRT) were dependent measures in a two (disease state: epileptogenic and non-epileptogenic) by two (laterality: right and left) analysis of variance. Analysis of co-variance (ANCOVA) was employed for the analysis of disease state (epileptogenic) controlling for clinical MRI findings (atrophy and non-atrophy). Linear regression models were used to investigate relationships between glutamate and

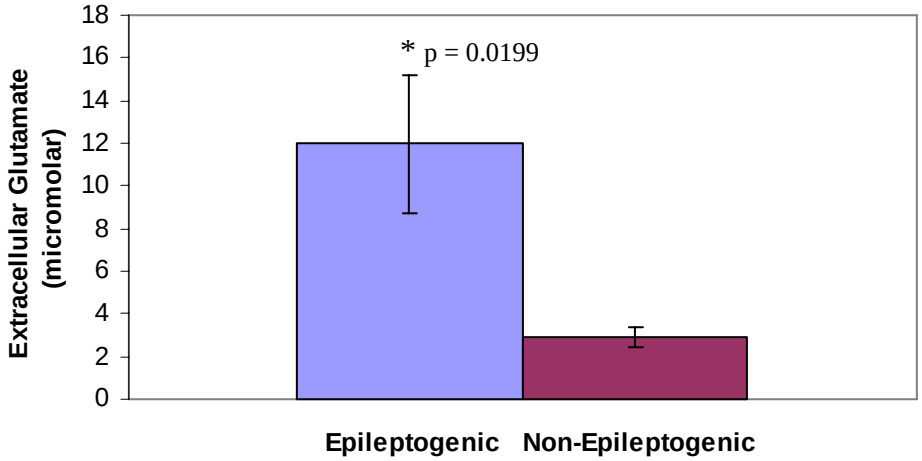
neuropsychological test performance based on *a priori* hypothesis. Significance level was set at 0.05. Data are reported as mean ± standard error of the mean. This investigator organized the dataset, and all statistical analyses were performed by I. Cavus, M.D., R. Gueorguieva, and B. Roach in conjunction with this investigator.

Results

Twenty-two patients were implanted with a total of 23 Spencer probes in the anterior hippocampus. Based on the intracranial EEG evaluation of spontaneous seizures, 14 hippocampal probes were classified as epileptogenic and 9 as non-epileptogenic. Of the 14 epileptogenic probes, 9 were located in the left hippocampus and 5 in the right hippocampus; 6 were in atrophic hippocampus, and 8 in non-atrophic hippocampus. Of the 9 non-epileptogenic probes, 5 were located in the left hippocampus and 4 in the right hippocampus; only 1 was located in atrophic hippocampus while 8 were in non-atrophic hippocampus (Table 1.)

The extracellular basal glutamate concentration from all microdialysis probes was estimated using the zero flow method and found to be 8.42 ± 10.47μM (mean ± SD), n = 23. The extracellular basal glutamate concentration in the non-epileptogenic hippocampus was 2.92 ± 1.37 μM (n = 9). In contrast, the basal glutamate concentration in the epileptogenic hippocampus was significantly greater (11.96 ± 12.24 μM, n = 14, p = 0.0199, t-test on log-transformed data) than in the non-epileptogenic hippocampus (see Fig 3). These results are consistent with prior studies that have demonstrated the epileptogenic hippocampus exhibits a higher basal level of glutamate compared to the non-epileptogenic hippocampus (69, 70). Since glutamate levels in the epileptogenic and non-epileptogenic hippocampus are significantly different, and our a *priori* hypothesis predicts that the glutamate levels in the epileptogenic hippocampus may impair cognitive performance, we examined the relationship between glutamate and the neuropsychological tests in the epileptogenic and non-epileptogenic hippocampus separately.

Fig 3. Basal extracellular glutamate levels in the epileptogenic and non-epileptogenic hippocampus.

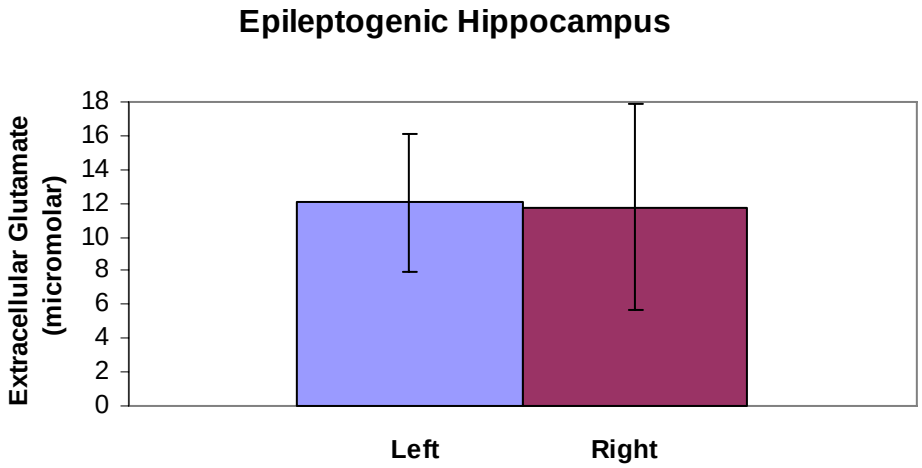


Basal glutamate levels in the epileptogenic hippocampus are significantly higher than basal glutamate levels in the non-epileptogenic hippocampus. ($p < 0.05$)*

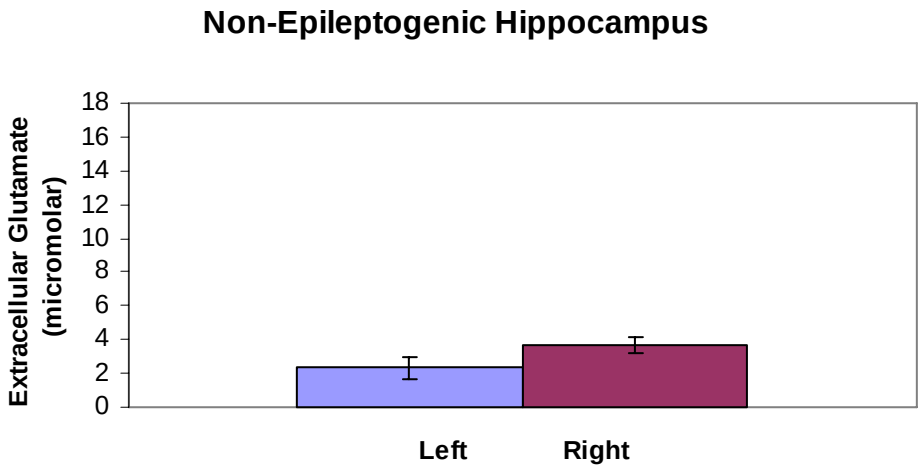
There has been considerable evidence suggesting that right and left hippocampus play different roles in cognitive functioning (17), therefore we have analyzed the effects of laterality within the epileptogenic and non-epileptogenic hippocampus. Within the epileptogenic probes, the basal glutamate level from the left hippocampus (12.06 ± 12.23 µM, mean ± SD, n = 9) did not differ significantly from the right hippocampus (11.78 ± 13.71 µM, n = 5), $p > 0.05$, t-test on log-transformed data). Similarly, no difference was found between the non-epileptogenic probes in the left hemisphere (2.34 ± 1.47µM, n = 5) and right hemisphere (3.64 ± 0.92µM, n = 4, $p > 0.05$, t-test on log-transformed data). (See Figure 4, A-B.)

Fig 4, A-B. Basal extracellular glutamate levels in the left and right epileptogenic and non-epileptogenic hippocampus.

A



B

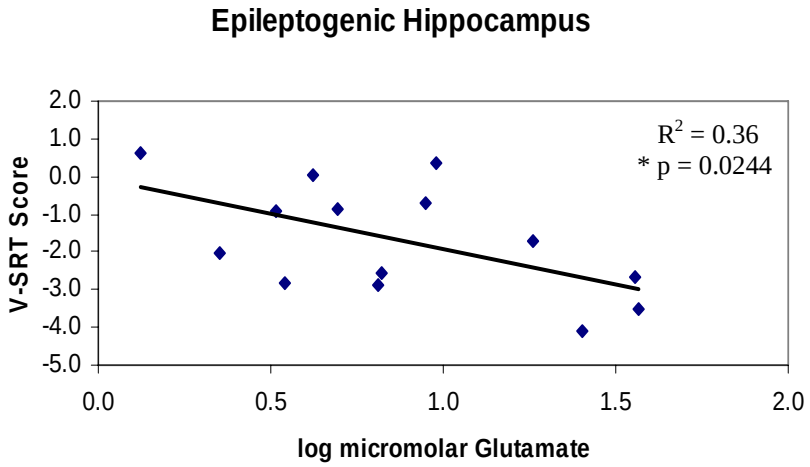


The basal extracellular glutamate levels are comparable ($p > 0.05$) within the (A) epileptogenic hippocampus and (B) non-epileptogenic hippocampus.

Further analysis was conducted to examine the effect of laterality on the relationship between individual neuropsychological assessment scores (VIQ, PIQ, FSIQ, V-SRT, and VM-SRT) and basal glutamate levels in the epileptogenic and non-epileptogenic probes, using a linear regression model. Within the epileptogenic probes, there were no significant correlations between basal glutamate concentrations and all neuropsychological measures for the right and the left hippocampus ($p > 0.05$ for all comparisons.) Of note, some results (VIQ, PIQ, and FSIQ) for the right hemisphere were bordering statistical significance (VIQ: $n = 6$, $R^2 = 0.6139$, $p = 0.0652$; PIQ: $n = 6$, $R^2 = 0.6113$, $p = 0.0662$; FSIQ: $n = 6$, $R^2 = 0.6581$, $p = 0.0501$). This finding may be spurious due to our small sample size, however the elucidation of laterality would benefit from further investigation in larger studies. Within the non-epileptogenic probes, there were no significant correlations between the basal glutamate levels and the neuropsychological measures of VIQ, FSIQ, V-SRT, and VM-SRT for the right and the left hippocampus ($p > 0.05$). However, only the extracellular levels in the left non-epileptogenic hippocampus correlated positively with the PIQ score ($n = 7$, $R^2 = 0.6662$, $p = 0.0251$). We are not sure about the significance of this finding, as it was unexpected. It is possible that this result within the healthy hippocampus may result from our small sample size. Conversely, increases in glutamate within physiologic ranges in the non-epileptogenic hippocampus may represent cognitive activation, which correlates with improved performance on the PIQ. For this reason, further investigation may beneficial to clarify this finding. Based on these data demonstrating little statistical significance between right and left hippocampal probes within both epileptogenic and non-epileptogenic probes, laterality effects will not be included during further analysis in this study.

Given evidence that elevated glutamate levels can be neurotoxic (7-9) and are associated with hippocampal neuronal loss and volume reduction (Cavus, unpublished data), and that these hippocampal pathologies are associated with verbal memory impairment (42-49), we sought to investigate whether elevated baseline glutamate levels in the epileptogenic hippocampus are associated with poorer cognitive function as compared to the non-epileptogenic hippocampus. The extracellular glutamate levels in the epileptogenic hippocampus correlated inversely with neuropsychological performance scores only on the V-SRT ($R^2 = 0.36$, $p = 0.0244$) (see Figure 5), but not for other tests (VIQ, PIQ, FSIQ, VM-SRT) ($p > 0.05$, linear regression model). This result is consistent with our hypothesis; an increase in basal glutamate levels to abnormally high levels is correlated with impaired hippocampal functioning measured by lower scores on the V-SRT, which is a test specific for hippocampal function. However, elevated basal glutamate levels in the epileptogenic hippocampus do not correlate with decreased scores on other neuropsychological assessments (VIQ, PIQ, FSIQ, and VM-SRT), which are less specific measures of hippocampal function. In the non-epileptogenic hippocampus where basal glutamate levels lie within normal ranges, there were no significant correlations between extracellular glutamate levels and neuropsychological performance scores for any measure (VIQ, PIQ, FSIQ, V-SRT, VM-SRT) ($p > 0.05$ for all correlations). This finding is consistent with our hypothesis given that physiologic levels of extracellular glutamate are not expected to impair cognitive processing, therefore further analysis within this study will be confined to the epileptogenic probes.

Figure 5. The relationship between basal extracellular glutamate levels in the epileptogenic hippocampus and V-SRT scores.

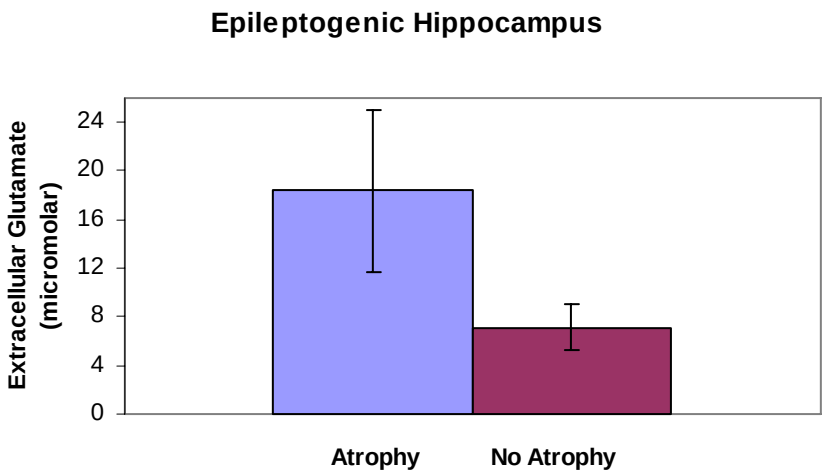


Basal glutamate levels and performance scores on the V-SRT are significantly and inversely correlated within the epileptogenic hippocampus, where the extracellular glutamate levels are abnormally high. Log transformation data were plotted according to the linear regression model. ($p < 0.05$)*

Thus far, our analysis of the relationship between basal glutamate levels and cognitive function has included all epileptogenic probes. In light of evidence suggesting that MRI-detected hippocampal atrophy is associated with decreased performance on neuropsychological assessment of verbal memory (47-49), we sought to clarify the relationship between glutamate levels and cognitive function among epileptogenic probes by controlling for the presence or absence of hippocampal atrophy. Of the 14 epileptogenic probes, 6 were located in atrophic hippocampus and 8 in non-atrophic hippocampus (Table 1). Within the epileptogenic probes, the basal glutamate level (mean ± SD) in atrophic hippocampus (18.36 ± 16.30μM, n = 6) is much greater than in non-atrophic hippocampus (7.15 ± 5.19μM, n = 8). However, this difference did not reach statistical significance ($p > 0.05$, t-test on log-transformed data), which is likely the result

of our small sample size (see Figure 6). Within the non-epileptogenic probes, the basal glutamate level (mean ± SD) from non-atrophic hippocampus was 2.92 ± 1.46μM (n = 8). Only one probe was located in the atrophic hippocampus, thus precluding further analysis within this group.

Fig 6. Basal extracellular glutamate levels in the atrophic and non-atrophic epileptogenic hippocampus.



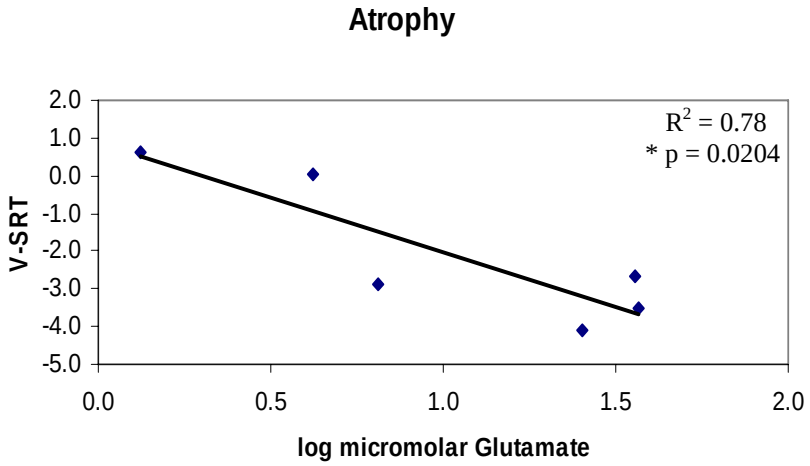
Within epileptogenic hippocampus, basal glutamate levels in atrophic hippocampus were much higher than in non-atrophic hippocampus. However this difference did not reach statistical significance ($p > 0.05$), likely due to small sample size.

Further analysis was conducted to examine the effect of atrophy on the relationship between basal glutamate levels and individual neuropsychological assessment scores (VIQ, PIQ, FSIQ, V-SRT, and VM-SRT) within epileptogenic probes. Analysis of co-variance (ANCOVA) test was utilized with atrophy as a co-variant, basal glutamate levels as predictor, and neuropsychological test results as outcome, followed by linear regression analysis. The relationship between glutamate levels and V-SRT

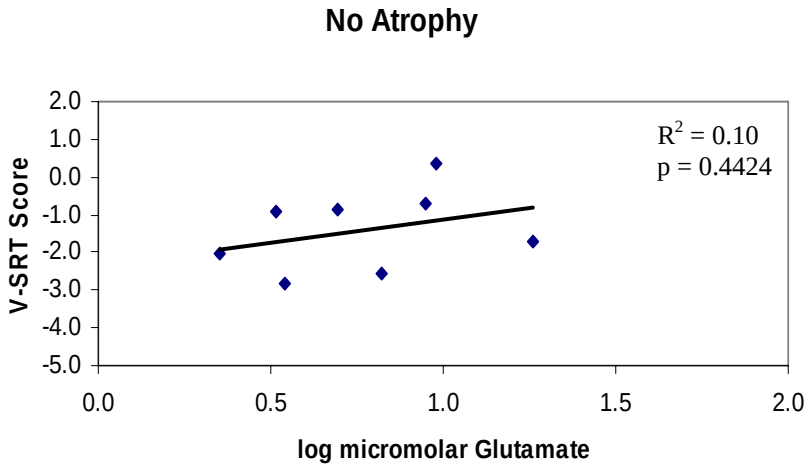
scores has been demonstrated to be significant within all epileptogenic probes; however when controlling for the presence of atrophy on ANCOVA tests it is revealed that this relationship is significant only within atrophic hippocampus ($F(1,5) = 13.89$, $p = 0.0204$), but not within non-atrophic hippocampus ($F(1,7) = 0.68$, $p = 0.4424$) (see Figure 7, A-B). In addition, although glutamate levels did not correlate significantly with the PIQ scores in the epileptogenic hippocampus, when controlled for the presence of atrophy, the glutamate levels correlated inversely with the PIQ scores within atrophic hippocampus ($R^2 = 0.7324$, $p = 0.0297$), but not within non-atrophic hippocampus ($R^2 = 0.2303$, $p = 0.2288$) (see Fig 7, C-D). These results indicate that within atrophic hippocampus, elevated basal glutamate levels are associated with significantly decreased cognitive performance as measured by both the V-SRT and PIQ tests. There were no significant interactions with atrophy as a co-variant for any of the other neuropsychological measures (VIQ, FSIQ, and VM-SRT, $p > 0.05$).

Figure 7, A-D. The relationship between basal extracellular glutamate levels in the atrophic and non-atrophic epileptogenic hippocampus and performance scores on V-SRT and PIQ tests.

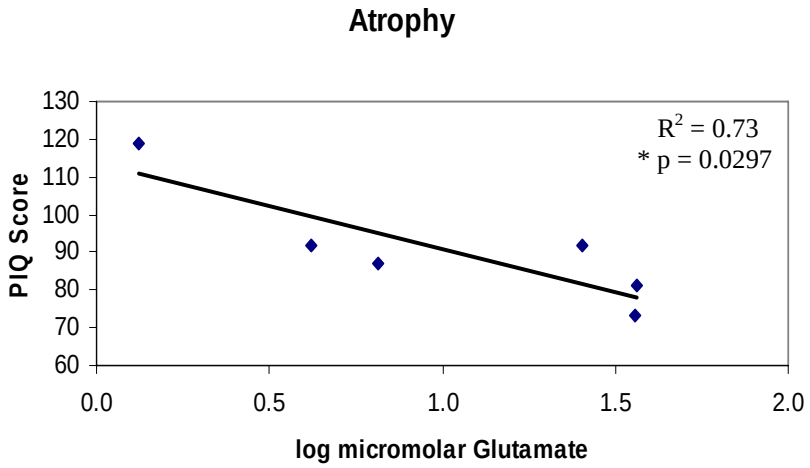
A



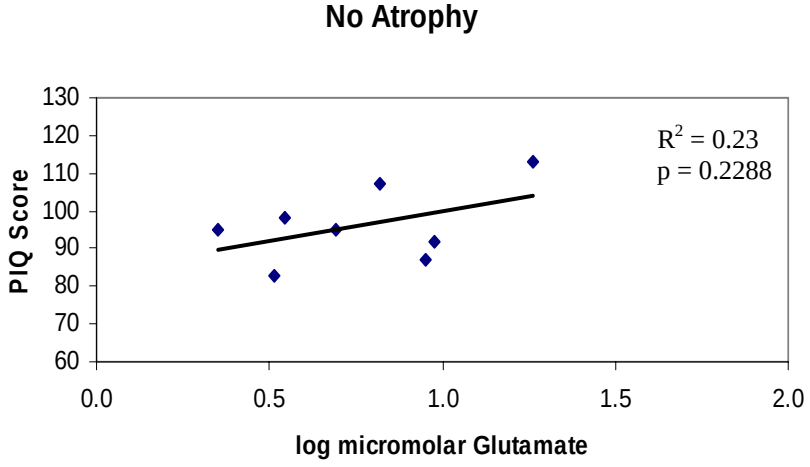
B



C



D



Within epileptogenic hippocampus, the increase in the basal glutamate level in the atrophic hippocampus, but not the non-atrophic hippocampus, correlated significantly with lower performance on the V-SRT test (A and B) and on the PIQ test (C and D). Log transformed glutamate data is plotted against the cognitive task scores according to linear regression model. ($p < 0.05$)*

Discussion

This study explores the relationship between the extracellular glutamate level in the hippocampus and the cognitive performance of patients with medication-resistant localization-related epilepsies. We used the zero-flow microdialysis method to estimate the basal extracellular concentration of glutamate in the epileptogenic and non-epileptogenic hippocampus of conscious neurosurgical epileptic patients during the interictal period (Table 1). The epileptogenic hippocampus had high basal glutamate levels (mean, 11.96μM; in some cases exceeding 35μM) which far exceeds neurotoxic glutamate levels of 5 micromolar or greater (82-84). In contrast, the non-epileptogenic hippocampus maintained low glutamate levels (mean, 2.92μM), which were within the range of previous studies in humans (69) and animals (85). We argue that the elevated basal glutamate levels in the epileptogenic hippocampus may be responsible for impaired cognitive functioning in patients with temporal lobe epilepsy.

Previous studies have reported that elevated glutamate levels are associated with cell loss and hippocampal atrophy in animals (86-88) and humans (Cavus, unpublished data), and that both cell loss and hippocampal atrophy are associated with verbal memory impairment (42-49). Our data demonstrate that elevated basal glutamate levels are associated with decreased performance on certain hippocampal cognitive tests. Specifically, elevated basal glutamate levels in the epileptogenic hippocampus are correlated with impaired verbal memory measured by the V-SRT, which is a neuropsychological assessment tool specific for hippocampal verbal functioning (44) (see Figure 5). Furthermore, when controlling for the presence or absence of MRI-detected hippocampal atrophy within epileptogenic regions, our study reveals that elevated basal

glutamate levels within atrophic hippocampus are correlated with decreased cognitive functioning measured by both the V-SRT and PIQ tests, but not within non-atrophic hippocampus (see Figure 7, A-D). No associations were elucidated between elevated basal glutamate levels in both atrophic and non-atrophic epileptogenic hippocampus and impaired cognitive performance measured by the VIQ, FSIQ, and VM-SRT. In the non-epileptogenic hippocampus, no associations were found between glutamate levels and performance on neuropsychological tests in agreement with our hypothesis. Basal glutamate levels remained low within this group, suggesting that the synaptic release and reuptake of glutamate is predominantly intact. Thus, it is reasonable to suggest that the activity-dependent synaptically-released glutamate is contained mostly within the synaptic cleft, and the low levels of extrasynaptic glutamate do not interfere with the glutamatergic signal processing that mediates cognitive functions (6).

Our data correlating elevated basal glutamate levels with impaired cognition do not imply causation. However, they are consistent with our proposed mechanism of neuropathology in which neurotoxic levels of glutamate damage the hippocampus and consequently impair cognitive functioning. Within epileptogenic hippocampus, glutamate concentrations may rise to neurotoxic levels as a result of impaired reuptake, excess synthesis and release, or impaired glutamate-glutamine cycling (69). Excessive concentrations of glutamate are known to produce excitotoxic cell death (7, 9), which may impair cellular functioning at the synaptic level, as well as mediate hippocampal cell loss and MRI-detected pathology (46, 47). We found that within epileptogenic probes, correlations existed between elevated glutamate levels and decreased cognitive processing for the atrophic hippocampus, but not for the non-atrophic hippocampus (see

Figure 7, A-D). This suggests that atrophy alone is not sufficient for impaired cognitive processing, but may require the concomitant elevation of glutamate concentrations. We therefore propose that the aforementioned cognitive deficits occur as a result of elevated glutamate levels with resultant neurotoxicity, and not simply from the presence of hippocampal cell loss and atrophy.

The association between elevated basal glutamate levels in the epileptogenic atrophic hippocampus and decreased scores on the V-SRT is consistent with our hypothesis, as this measure has been previously demonstrated to be specific for pyramidal cell loss (44). However, the association between elevated glutamate and decreased performance on the PIQ was unexpected, as this test is a measure of overall intelligence which depends primarily upon global cognitive processes (41), and is not sensitive for hippocampal pathology (42). This finding may be spurious given our small sample size (n = 6). Alternately, although PIQ measures nonverbally-mediated global cortical function (42), the subtests of block design and object assembly measure aspects of visual-spatial ability, to which the hippocampus is believed to contribute. Numerous studies have demonstrated hippocampal involvement in the encoding and retrieval of visual-spatial memory in animals (24, 89-91). For example, rodents with specific lesions of the hippocampal formation demonstrate impairments in navigating to a goal location (89-91). Defining the hippocampal role in visual-spatial memory within humans has been more complicated, since the method of memorizing a spatial location to reach a goal has been unique to rodent models (92). However, in a recent functional magnetic resonance imaging (fMRI) study in humans, Astur and colleagues (92) demonstrated hippocampal activation occurs during the performance of a computer-generated virtual

reality 8-arm radial maze, thus mimicking the design of rodent studies. Furthermore, the hippocampus has been implicated in other non-verbally mediated cognitive tasks in human fMRI studies, including face recognition (93) and picture recognition (94). Given the role of the hippocampus in visual-spatial memory processing, elevated glutamate levels within the atrophic epileptogenic hippocampus may result in neuronal damage and subsequent impairment in visual-spatial learning, as detected by the PIQ. However, further studies will be necessary to clarify the significance of this finding.

No associations were found between glutamate levels and cognitive performance on measures of VIQ and FSIQ; this is consistent with our hypothesis given the V-SRT is more dependent upon hippocampal function than the VIQ (44), and the FSIQ is a composite score to which the VIQ contributes (41). The VM-SRT measures visual-spatial memory which is believed to be a non-dominant (usually right) hippocampal-dependent task (41), however our results are not entirely consistent with this, as elevated glutamate levels do not impair function on this test. We would expect that epileptogenic, right-sided, atrophic hippocampus would have elevated basal glutamate levels which might correlate with impaired VM-SRT performance. However, only 3 patients within this study fulfill these criteria (Table 1); this small patient sample size may preclude emergence of statistical significance, therefore future studies are warranted.

Our study sample consists of patients who are left hemisphere dominant for language as measured by the intracarotid amobarbital procedure. As the dominant hippocampus has been implicated in verbal memory and the non-dominant hippocampus with visual-spatial learning (17), we would expect elevated glutamate levels in the left-sided epileptogenic hippocampus to be associated with decreased performance on the V-

SRT, and the right-sided epileptogenic hippocampus with impaired visual-spatial testing. However, our results do not demonstrate significant effects regarding laterality. Of note, in the right (non-dominant) hemisphere, associations between glutamate levels and VIQ, PIQ, and FSIQ scores were bordering significance. In addition, within healthy left-sided hippocampus where glutamate levels are maintained at physiologic levels, a significant correlation exists between glutamate levels and PIQ scores. These data may be spurious given our small sample size, however the effects of laterality would benefit further investigation in studies involving more subjects.

Our study was confined to probes located in the anterior hippocampus, as mesial temporal lobe epilepsy preferentially involves the anterior mesial lobe and intraoperative probe placement was determined by clinical suspicion of seizure focus. Prior studies have reported differential involvement of the anterior and posterior hippocampus in cognitive processing (24, 26), such that the anterior hippocampus is believed to be involved in memory encoding of novel stimuli, while the posterior hippocampus is believed to mediate memory retrieval, familiar information processing, and verbal and spatial memory (25, 27-32). In light of these prior studies, we would not expect to see a robust correlation between glutamate levels in the anterior hippocampus and verbal memory tests. However, we noted a significant correlation ($R^2 = 0.7764$, $p = 0.0204$) between elevated glutamate concentrations in the epileptogenic atrophic hippocampus and verbal memory measured by the V-SRT. This suggests that verbal information processing is not confined to the posterior hippocampus, but may also involve anterior regions. In addition, the heterogeneous function of the hippocampus along its anterior-posterior axis predicts that little visual-spatial cognitive activity would occur in the

anterior hippocampus. This may contribute to our data demonstrating no significant correlation between glutamate levels in the anterior epileptic atrophic hippocampus and visual-spatial activity measured by the VM-SRT. Furthermore, our small sample size has precluded examination of both anterior and posterior hippocampus, thus the selective effects of glutamate levels within the anterior versus posterior hippocampus on cognitive functioning would benefit further study employing a larger sample size.

Confounding Factors

There are several limitations to the present study. First, the technique of intracerebral microdialysis was used to estimate extracellular glutamate concentrations in the conscious human hippocampus. It is important to note that microdialysis sampling reflects a pooled measure of extracellular fluid in the immediate vicinity of the catheter, which may differ considerably from intra-synaptic concentrations of neurotransmitters. The small sampling tissue volume of the microdialysis may not reflect the overall chemical milieu in the hippocampus, where larger regions may be involved in a given cognitive task. Nonetheless, microdialysis provides the closest *in vivo* look at neurobiological activity at the extracellular space in the awake human brain and permits the investigation of cognition at the level of neurotransmitters. Second, because the investigational procedure employed in this study involves an invasive intracranial surgical procedure within a specific subset of patients with medically refractory epilepsy, there is a paucity of patients available for study, even in a large epilepsy center over the course of 5 years. The small patient size examined in this investigation may have prevented the emergence of statistically significant results that would have been realized

in a larger study, and may have resulted in spurious results that would not be significant within a greater sample size. Finally, this study is designed to examine the relationship between glutamate levels in epileptogenic and non-epileptogenic hippocampus and cognitive function measured by neuropsychological tests. It would be presumably very difficult to control for the possible patient who has an intracranial probe in non-epileptogenic (and presumably healthy) hippocampus, but has an additional vigorously epileptogenic hippocampal seizure focus not captured by probe sampling and which may impair cognitive function, thus generating inaccurate correlations.

Clinical Implications

Within neuropsychiatry, there exists an abundance of clinical disorders associated with hippocampal structural abnormalities. As reviewed by Astur and colleagues (92), patients with schizophrenia and post-traumatic stress disorder often have smaller hippocampus than aged-matched controls, while patients with Alzheimer's disease may experience the earliest signs of neurodegeneration within the hippocampus followed by subsequent development of amyloid plaques and neurofibrillary tangles. Mesial temporal lobe epilepsy patients suffer most commonly from mesial temporal sclerosis, characterized by hippocampal atrophy and gliosis (51, 52). Given the pivotal role of the hippocampus in learning and memory, these disorders occur concomitantly with deficits in cognitive functioning. The present study demonstrates the association between elevated glutamate levels and impairment of certain forms of cognitive functioning in the setting of hippocampal atrophy. The abnormally enhanced glutamatergic activity may result in excitotoxic cell damage and death to hippocampal structures, thus serving as a

possible mechanism of neuropathological injury and subsequent cognitive impairment. Meaningful clinical implications may therefore exist for potential treatment options of diseases mediated by glutamate neurotoxicity and subsequent hippocampal atrophy. Specifically, lowering the basal extracellular glutamate with medications may help to alleviate neuronal cell loss and sclerosis and preserve cognitive functioning. In agreement, glutamate-receptor antagonists have been shown to possess both anticonvulsant and neuroprotective properties (70).

To our knowledge, this is the first study to demonstrate the association between basal glutamate levels in conscious humans undergoing microdialysis and baseline neuropsychological functioning. However, as our sample size was small (n = 22), future investigation is warranted to clarify points where our data revealed borderline statistical significance (i.e., glutamate levels in the right epileptogenic hippocampus versus PIQ, VIQ, and FSIQ), lack of significance where meaningful relationships were expected conceptually (i.e., glutamate levels in the right epileptogenic hippocampus versus VM-SRT), and statistical significance where it was not initially hypothesized (i.e., glutamate levels in the epileptogenic atrophic hippocampus versus PIQ). Although effects of laterality are not demonstrated in the present report, this finding is likely the result of our small sample size and would benefit further investigation in a larger study. Furthermore, additional study exploring glutamate levels within both the anterior and the posterior hippocampus would be helpful to elucidate the function of the hippocampus along its longitudinal axis, as compared to neuropsychological measures.

Conclusion

In summary, glutamate is the principal excitatory neurotransmitter and levels remain low in healthy brain, however concentrations may rise to neurotoxic levels in the epileptogenic hippocampus (1, 5, 7, 64-70). The hippocampus is responsible for learning and memory (15-17, 24-27), thus impaired hippocampal function in TLE is associated with cognitive deficits (40, 54, 55). Specifically, microdialysis evidence demonstrates that elevated glutamate levels are associated with cell loss and hippocampal atrophy (Cavus, unpublished data), and previous studies have demonstrated that both cell loss and hippocampal atrophy are associated with verbal memory impairment (42-49). Our data demonstrate that basal glutamate concentrations within the epileptogenic hippocampus reach neurotoxic levels and are significantly higher than the non-epileptogenic hippocampus. Elevated basal glutamate levels in the epileptogenic hippocampus are associated with decreased verbal memory performance measured by the V-SRT. When controlling for atrophy, elevated basal glutamate levels in the epileptogenic hippocampus are associated with impairment on V-SRT and PIQ performance, but not in epileptogenic non-atrophic hippocampus. We propose that the aforementioned cognitive deficits occur as a result of elevated glutamate levels with resultant neurotoxicity and subsequent hippocampal pathology. This mechanism of injury may occur in epilepsy and other clinical disorders marked by hippocampal pathology such as schizophrenia, post-traumatic stress disorder, and Alzheimer's disease. This provides the opportunity for treatment options aimed to medically maintain intrahippocampal glutamate at low levels to prevent neurotoxicity, which may in turn help patients preserve their cognitive function.

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